Like Sugar for Poison: Glucose as a Substitute for Benzene

Joseph Tart / Olivia James

Scientists searching for a replacement for toxic substances in chemical manufacturing may have come up with a sweet solution: glucose.

Glucose, the body's main fuel, is found in certain foods and also formed by the breakdown of sugars and starches. It may one day be a replacement for benzene, a highly regulated compound that is ubiquitous in the chemical industry (12 billion pounds were produced in the United States in 1993). Benzene helps make jeans blue—it's the feedstock for indigo dye—and ice cream vanilla flavored—it's the source of vanillin. It's also the starting point for a number of important industrial chemicals including hydroquinone, used in film developing, phenol, used to make solvents, and adipic acid, which is used to make nylon. Benzene is also a potent carcinogen.

"Benzene is quite toxic. It's nobody's friend. Companies don't like it because they have to comply with [benzene] regulations, and they're expensive. Chemists just don't like to handle it. Whereas with glucose, nobody has any concerns about it," says Stephen DeVito of the EPA's Office of Pollution Prevention and Toxics.

Living Catalysts

The basic research on replacing benzene with glucose is being carried out at Michigan State University by chemist John Frost, whose work is funded primarily by the EPA and the National Science Foundation.

Frost's research is an example of "green chemistry," an attempt to incorporate environmental concerns into the chemical manufacturing process. It focuses on creating processes that avoid toxic emissions and harmful by-products. Glucose-based chemistry, says Frost, needs only water and temperatures typically no higher than body temperature. Benzene-based processes, on the other hand, are energy-intensive and demand high temperatures.

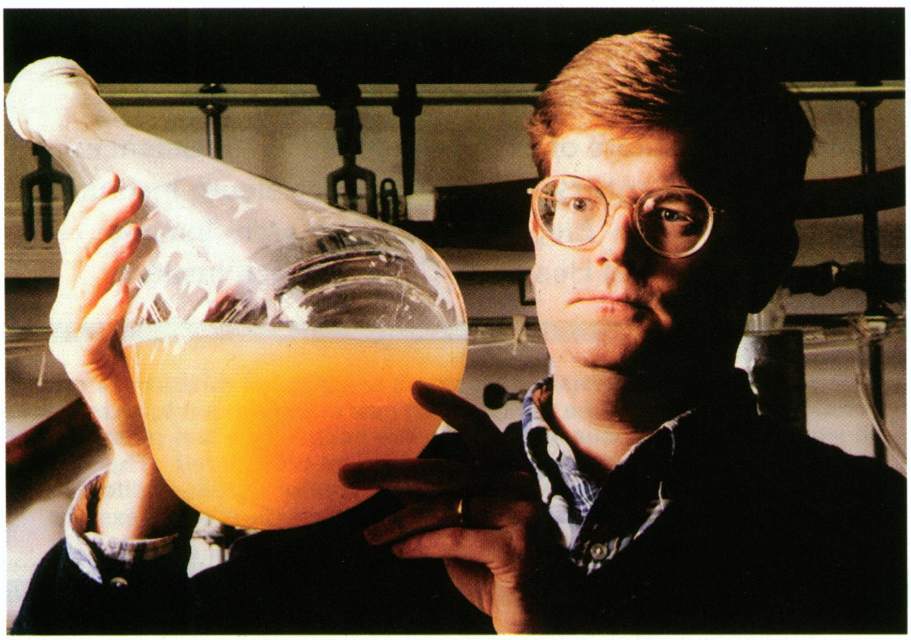
The process of substituting glucose for benzene involves biocatalysis: using bacteria to make chemicals. It capitalizes on the fact that both benzene and glucose have similar chemical structures—they are both carbon-ring compounds, explains Donald Paul, president of Bio-Technical Resources, a genetic engineering firm in Manitowoc, Wisconsin.

Frost mixed bioengineered *E. coli* bacteria with glucose to replace the link in the chemical reaction that uses benzene to make adipic acid. "What you can do by mixing genes from one organism to the next is to create pathways, which have bits and pieces of other pathways, but in their entirety don't exist in nature," said Frost.

To make adipic acid conventionally, benzene is hydrogenated to yield cyclohexane, which is then oxidized in the presence of metal catalysts to produce a mixture of cyclohexanone and cyclohexanol. When the mixture is oxidized with nitric acid, it produces adipic acid and releases nitrous oxide, an environmentally harmful chemical.

To provide a more benign way to produce adipic acid, Frost inserted genes from two bacteria, *Klebsiella pneumoniae* and *Acinetobacter calcoaceticus*, into *E. coli*. *E. coli* converts glucose into 3-dehydroshikimate (DHS), which is a normal product in the process of making amino acids. The added genes produce enzymes, one of which, in effect, hijacks DHS from amino acid formation, and two others which help convert it to *cis,cis*-muconic acid, a constituent of adipic acid, which is hydrogenated to yield adipic acid. Unlike benzene, glucose contains oxygen atoms that eliminate the oxidation step, eliminating nitrous oxide emissions.

Using genes from *Klebsiella pneumoniae* and the same process, Frost has produced



Michigan State U.

Shaking things up. Researcher John Frost is putting a "green" twist on chemical manufacturing.

catechol, another important chemical produced by benzene. Catechol is used to make vanillin, some drugs, and agrichemicals. In this process, not only does glucose replace benzene, but the toxic chemical intermediate, phenol, is eliminated, as is corrosive hydrogen peroxide.

Hydroquinone, which is produced in the catechol-manufacturing process, can also be made using glucose. In this case, genes from *Klebsiella pneumoniae* are inserted into *E. coli*, allowing it to synthesize quinic acid. Oxidation with manganese produces benzoquinone, which can then be reduced to hydroquinone. Approximately 88 million pounds of hydroquinone are produced annually. Although this technology avoids the use of benzene, it produces manganese salts that can present environmental problems.

Ecology and Economics

Using benzene to produce adipic acid not only requires petroleum, a nonrenewable resource, it also spells trouble for the atmosphere. The manufacturing process produces carbon dioxide, a greenhouse gas. The nitrous oxide given off by the process also thins the stratospheric ozone layer and plays a role in global warming. Chemists Mark Thiemans and William Trogler of the University of California-San Diego reported in 1991 that production of adipic acid is responsible for one-tenth of the annual increase in atmospheric nitrous oxide. Four billion pounds of adipic acid are made globally each year, 1.75 billion of it in the United States, according to Frost.

"Glucose is renewable, and anything you make from starch or glucose is pulling CO_2 from the atmosphere. It's using plant-fixed carbon dioxide," says Frost.

Glucose is available from a variety of sources. While crops such as corn may be one source of glucose, plants like switchgrass and prairies grasses are also possible sources, as well as fast-growing hybrid poplar trees. "A lot of marginal land could be made productive for this type of application," says Frost. It would involve what he describes as "very low input agricultural techniques," meaning minimal use of chemical fertilizers and irrigation. Cellulose-containing waste products can also be used as glucose sources. "When farmers are done harvesting their crops, there's stubble left over. That is useful stuff," says Frost. And, according to Frost, the waste streams for the use of glucose as a starting material are no different from what an ordinary municipal sewage treatment plant handles.

Frost argues that stricter EPA regulations are a driving force in pushing chemical companies away from using benzene. Last year the EPA told chemical manufacturers they had three years to slash toxic air emissions by nearly 90% from 1990 levels. Benzene is one of the chemicals covered by the rule. The costs of eliminating these emissions is estimated between $450 million and $1 billion. While not all the costs can be attributed to benzene, this is another argument for an alternative.

"There are literally thousands of theoretical pathways toward making our complex chemical products," says Paul Anastas of EPA's Office of Pollution Prevention and Toxics. "Always the way of approaching chemical products is to the use lowest cost feedstock and convert as much as possible to product. What has not traditionally been a consideration is the substances generated as by-products or the inherent toxicity of those products. With the added costs of regulatory compliance, waste disposal, and waste treatment, the economic equation has changed. Rather than looking at simply the lowest cost feedstock in terms of highest percentage conversion into products, it will be economically beneficial to also consider the toxicity of feedstocks, by-products, and so forth, to lower all of these other costs such as regulatory compliance."

"[Frost is] setting the paradigms for this kind of work so hopefully in the future, when industry starts to think about going commercial with this type of thing, they can use this type of methodology," says George Rubottom, the National Science Foundation's program manager for research in synthetic organic chemistry.

Lauren Blum, a chemist with the Environmental Defense Fund, thinks it's a worthwhile approach. "Anytime you move from petrochemicals to biomass it should make sense from an energy perspective," she says. One concern she does have is ensuring that the energy demands to supply the glucose are not excessive.

Frost's research has generated some industrial interest and funding. The DuPont Company is partially funding his research, though David Anton, DuPont's research manger for bioprocessing development, won't divulge the amount. But he says that "DuPont has made a relatively major effort to look at this type of an area."

Even though DuPont announced in 1991 it would develop technology to capture and recycle nitrous oxide formed in making adipic acid, substituting glucose for benzene may still make economic sense. "The thing that drives every business is cost. Glucose has the potential to be cheaper than benzene. Benzene is thirteen cents per pound; glucose is five and a half to six cents," says Anton.

"What this really is, is an opportunity to make more money," says Allan Ford, a member of the American Chemical Society's Committee on the Improvement of the Environment. Ford, who now heads the Gulf Coast Hazardous Substance Research Center, a multi-university consortium based in Beaumont, Texas, was a former director of environmental science for Monsanto Company in St. Louis.

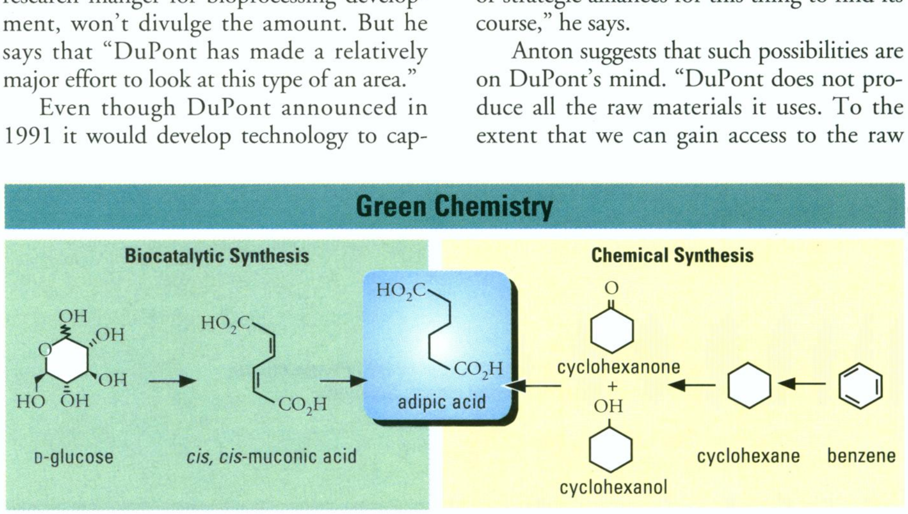
Surmountable Obstacles

Despite its promise, there are questions that may cloud the future of glucose as a bioengineered industrial feedstock. One problem that must be solved is how to use glucose on a large scale. Frost has been able to produce "shake flask" amounts of adipic acid in the laboratory, in the range of three to five grams. Obviously, far larger amounts will be required by industry. Even though drug firms produce insulin with genetically engineered bacteria, nothing of the size required to produce vast amounts of industrial chemicals using bacteria has been done before, says DeVito.

It will take some convincing to show people the advantages of Frost's technology, says Anastas. These advantages include less waste and a cleaner way of achieving the end product.

Frost, who optimistically describes the adoption of such technology as "inevitable," notes that its use will require changes in the way chemical companies do business. "The companies that are basic in glucose feedstock are not basic in chemicals. Chemical companies don't know anything about fermentation. So you have to have acquisitions or strategic alliances for this thing to find its course," he says.

Anton suggests that such possibilities are on DuPont's mind. "DuPont does not produce all the raw materials it uses. To the extent that we can gain access to the raw



John W. Frost

materials that we need to run our processes in an economically favorable way, glucose will be another raw material that we will go after," he says.

Marion Bradford, a research scientist at A. E. Staley Manufacturing Company of Decatur, Illinois, says Frost's work has attracted interest from grain processing companies such as Staley.

Another question is how much benzene could this new technology replace? Frost estimates about 20%, pointing to adipic acid as the major product glucose could be used to make. Said Anastas, "I do think replacing a couple of billion pounds of benzene is significant, especially when you consider the workers who have to deal with this carcinogenic material."

DuPont's Anton is more cautious. "We believe there will be cases where glucose will displace petroleum as the preferred feedstock for some chemical applications. We don't know what those are going to be because the way things will ultimately play out, we'll find in some cases it will be cheaper with glucose, in other cases it will be cheaper with petroleum." And if the cost of glucose is low enough the technology could "offer some relief from benzene in some applications," says David Kurtz, professor of chemistry at Ohio Northern University.

Frost himself acknowledges that the current methods of making adipic acid are cheaper than a glucose-based technology. But he argues that factoring in costs of controlling nitrous oxide and benzene emissions will make the new technology more appealing.

"It really is the front end of a whole philosophy of developing a manufacturing system that has minimal environmental impact," says Ford. "I think there are real possibilities, once you start recognizing that damaging the environment costs money."

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Suggested Reading

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